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# Akermanite used as an alkaline biodegradable implants for the treatment of osteoporotic bone defect

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ABSTRACT

In osteoporosis scenario, tissue response to implants is greatly impaired by the deteriorated bone regeneration microenvironment. In the present study, a Mg-containing akermanite (Ak) ceramic was employed for the treatment of osteoporotic bone defect, based on the hypothesis that both beneficial ions (*e.g.* $Mg^{2+}$ *ect.*) released by the implants and the weak alkaline microenvironment pH (μe-pH) it created may play distinct roles in recovering the abnormal bone regeneration by stimulating osteoblastic anabolic effects. The performance of Ak, β-tricalcium phosphate (β-TCP) and Hardystone (Har) in healing a 3 mm bone defect on the ovariectomized (OVX) osteoporotic rat model was evaluated. Our results indicated that, there's more new bone formed in Ak group than in β-TCP or Har group at week 9. The initial μe-pHs of Ak were significantly higher than that of the β-TCP and Blank group, and this weak alkaline condition was maintained till at least 9 weeks post-surgery. Increased osteoblastic activity which was indicated by higher osteoid secretion was observed in Ak group at week 4 to week 9. An intermediate layer which was rich in phosphorus minerals and bound directly to the new forming bone was developed on the surface of Ak. In a summary, our study demonstrates that Ak exhibits a superior bone regenerative performance under osteoporosis condition, and might be a promising candidate for the treatment of osteoporotic bone defect and fracture.


## 1. Introduction

Osteoporosis is a multifactorial skeletal disease characterized by low bone mass with deteriorated bone microstructure [1]. Compared with normal bone, the regeneration process of osteoporotic bone defect was strongly impaired due to the weaker capability of bone formation than bone resorption. Therefore, in the application of biomaterials to osteoporotic patients, a proper physical support together with the function to ameliorate the unbalanced regeneration microenvironment are strongly recommended [2].

Among various influencing factors, the *in vivo* microenvironment pH (μe-pH) has been demonstrated to be important in the bone defect rehabilitation process [3–5]. According to our previous studies, the detection of μe-pH was realized by using a pH microelectrode, and the results indicated the μe-pHs of our specifically tested biomaterials were significantly different from the homogeneous peripheral blood pH and exhibited unique changing patterns with time. The alkaline biodegradable material showed promising healing effect in the context of osteoporotic bone defects [5]. Besides, we realized that the μe-pH generated by implant biodegradation may be influenced by the release of surface ions, such as $Si^{4+}$, $Mg^{2+}$, and $Zn^{2+}$, so that a similar μe-pH could be generated through a combination effect of different released ions. Although the beneficial effects of these ions have been widely reported [6–9], the regulation and function of the *in vivo* μe-pH change, according to our literature review, are still remained to be further discussed.

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Calcium silicate ($CaSiO_3$, CS) is celebrated for its bioactivity, osteoinductivity and biodegradability which could grant a better bone regenerative capacity than β-tricalcium phosphate (β-$Ca_3(PO_4)_2$, β-TCP) [10,11]. However, the problem of high dissolution rate limits the application of CS as an orthopaedic implants [12]. A Ca–Mg/Zn–Si bioceramic system generated by incorporating magnesium/zinc into silicate based framework (*e.g.* akermanite ($Ca_2MgSi_2O_7$, Ak), diopside ($CaMgSi_2O_6$), hardystonite ($Ca_2ZnSi_2O_7$, Har) *ect.*) are expected to exhibit a more controllable degradation rate [13] and suitable mechanical properties [14] for applications as orthopaedic biomaterials. *In vitro* study revealed that adipose-derived stem cells and osteoblasts presented better proliferation and osteogenesis behavior on akermanite than on β-TCP [15,16]. Consistently, a faster new bone formation rate derived from non-osteoporotic rabbit femur bone defect was observed in akermanite porous bioceramic than in β-TCP [17]. A recent research further proved that akermanite showed promotion effects on angiogenesis while suppress osteoclastogenesis for osteoporotic bone regeneration [18].

However, although the combination effect of $Mg^{2+}$ and $Si^{2+}$ in akermanite on osteogenesis under osteoporotic condition has been reported, to our knowledge, there's currently a lack of study that is focused on the evaluation of the *in vivo* μe-pH change influenced by the release of these ions. Besides, knowledge of element distribution of Ak in microenvironment between implant and new bone is still in deficiency. In this study, a Mg-containing akermanite has been fabricated and applied under osteoporotic bone defect regeneration condition, combining the repair capacity of beneficial ions and alkaline μe-pH. The simulative effect of akermanite on new bone was examined by an OVX rat tibia defect model, and the interfacial elements distribution between implant and new bone was examined by energy-dispersive X-ray spectroscopy (EDX) linear scanning.

## 2. Materials and methods

### 2.1. Materials characterization

Akermanite ($Ca_2MgSi_2O_7$, Ak), Hardystone ($Ca_2ZnSi_2O_7$, Har), and beta-tricalcium phosphate (β-$Ca_3(PO_4)_2$, β-TCP) were kindly provided by Shanghai Institute of Ceramics, Chinese Academy of Sciences. Briefly, akermanite and hardystonite were fabricated by sol–gel process with raw materials of tetraethyl orthosilicate ($(C_2H_5O)_4Si$), magnesium/zinc nitrate hexahydrate ($Mg(NO_3)_2\cdot 6H_2O$/$Zn(NO_3)_2\cdot 6H_2O$) and calcium nitrate tetrahydrate ($Ca(NO_3)_2\cdot 4H_2O$) [17]. β-TCP was prepared by the reaction of $Ca(NO_3)_2\cdot 4H_2O$ with $(NH_4)_2HPO_4$ [10]. All materials were ground and sieved to 300–450 μm (irregular shape), and sterilized by gamma irradiation (270 Gy) before use. The nature of the tested materials used in this study was confirmed by X-ray diffraction (XRD) spectrum with a D8 Advance (Bruker, Billerica, MA, USA). The 2θ was set from 10° to 80°.

### 2.2. Animal model

#### 2.2.1. Osteoporotic rat model

All animal surgical procedures were conducted under protocols approved by the Committee on the Use of Live Animals in Teaching and Research, The University of Hong Kong (CULATR No. 2572-11; 2555-11). Female Sprague-Dawley rats aged 10 months were chosen in this study. Osteoporosis was inducted by ovariectomy (OVX) surgery as previously described [19,20]. Briefly, after anesthetization, an incision was made at the midline of the abdomen through which both ovaries were excised bilaterally; bleeding control procedures were instituted and the incision was sutured. Bone mineral density (BMD) of the proximal tibia was measured by Micro-computerized tomography (CT) (Skyscan 1076, Skyscan, Kontich, Belgium). 3 months after OVX surgery, osteoporotic rat model was successfully established.

#### 2.2.2. Material implantation

A secondary surgery was performed three months after the OVX surgery, bilateral bone defects were created in the median aspect of the tibial shaft, below the tibial plateau. Briefly, incisions were made bilaterally after shaving and aseptic procedures on the median aspect of the proximal tibia. Defects with depth and diameter of 3 mm were created with a 3-mm drill at low speed. Both defects were then packed gently with each material powders (Ak, Har or β-TCP) with four replicates for each time point. After μe-pH detection, the entrance of the defect was sealed using bone wax (Ethicon, Somerville, NJ, USA) and the skin was sutured (Ethilon, Ethicon). Blank controls were treated similarly but without material implantation. Antibiotic (Baytril®, Enrofloxacin, Bayer HealthCare, Kiel, Germany) was administered in the drinking water for 3 days. Rats were euthanized with an overdose of pentobarbital (Alfasan; 150 mg/kg) at 1, 4 or 9 weeks. Both tibiae were then harvested.

#### 2.2.3. Detection of in vivo μe-pH

The pH meter was normalized before use (Model 60, Jenco, San Diego, CA, USA), and the μe-pH was determined immediately after materials implantation. The sensing tip of the microelectrode (MI-413P, Microelectrodes, Bedford, NH, USA) was placed on the surface of the blood-saturated packed powder, and the stable value of the initial detection was used for analysis. Protein contamination on the sensor was removed after each measurement by immersing the sensor in enzymatic detergent (Tergazyme, Alconox, White Plains, NY, USA). Before tissue was harvested, the bone wax was removed, the surface layers of the implants were carefully scraped with a scalpel to expose the implant fully, and thus its internal microenvironment, for its μe-pH to be measured again in the same way.

### 2.3. Evaluation

#### 2.3.1. Micro-CT analysis

The implantation sites were scanned by Micro-computerized tomography (CT) at a voltage of 88 kV and current of 100 μA. The rotation step was 0.6° and the isotropic voxel size was 17.33 μm. Data was reconstructed by software (NRecon Server, version 1.6.6.0, Skyscan). A column of 0.4 × 1.0 $mm^2$ (height × radius) in the center of the implantation site was chosen as the volume of interest (VOI). According to our results, the X-ray attenuation coefficient (AC) of rat trabecular bones was lower than 25.1 $m^{-1}$, so that objects with an AC higher than 25.1 $m^{-1}$, which were chosen as the object of interest, can be identified as the remaining implant. Volume of object in VOI (Obj.V/TV) and relative surface area (Obj.S/Obj.V) were calculated by software (CT Analyser, V. 1.10.0.1, Skyscan). The 3D VOI images at time points of week 1, 4 and 9 were created based on AC (CT Vol, version 2.1.0.0, Skyscan).

#### 2.3.2. Decalcified histology staining

Harvested tibias were processed for standard procedures of fixation, ethylene diamine tetraacetic acid (EDTA) decalcification, dehydration and paraffin-embedding [21]. Five-micrometer sections were created for histological staining (haematoxylin and eosin, Sigma–Aldrich) to detect the specific tissue response to the implanted materials. Semi-quantitative examinations for new bone formation were analyzed by 8 parallel samples, and the histogram was established by software (GraphPad Prism 5, La Jolla, CA, USA).